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RESEARCH PAPER

Detection of QTc interval prolongation using jacket telemetry in conscious non-human primates: comparison with implanted telemetry

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BACKGROUND AND PURPOSE

During repeat-dose toxicity studies, ECGs are collected from chemically or physically-restrained animals over a short timeframe. This is problematic due to cardiovascular changes caused by manual restraint stress and anesthesia, and limited ECG sampling. These factors confound data interpretation, but may be overcome by using a non-invasive jacket-based ECG collection (JET). The current study investigated whether a jacketed external telemetry system could detect changes in cardiac intervals and heart rate in non-human primates (NHPs), previously implanted with a PCT transmitter.

EXPERIMENTAL APPROACH

Twelve male cynomolgus monkeys were treated weekly with vehicle or sotalol (8, 16, 32 mg kg^{-1}) p.o. ECGs were collected continuously for 24 hours, following treatment, over 4 weeks. A satellite group of six NHPs was used for sotalol toxicokinetics.

KEY RESULTS

Sotalol attained C_{max} values 1–3 hours after dosing, and exhibited dose-proportional exposure. In jacketed NHPs, sotalol dose-dependently increased QT/QTc intervals, prolonged PR interval, and reduced heart rate. Significant QTc prolongation of 27, 54 and 76 msec was detected by JET after 8, 16, and 32 mg kg^{-1} sotalol, respectively, compared with time-matched vehicle-treated animals. Overall, JET-derived PR, QT, QTc intervals, QRS duration, and heart rate correlated well with those derived from PCT.

CONCLUSIONS AND IMPLICATIONS

The current findings clearly support the use of JET to quantify cardiac interval and rhythm changes, capable of detecting QTc prolongation caused by sotalol. JET may be a preferred method compared to restraint-based ECG because high-density ECG sampling can be collected in unstressed conscious monkeys, over several weeks.

Abbreviations

AP, arterial pressure; bpm, beats per minute; HR, heart rate; JET, jacketed external telemetry; NHP, non-human primates; QTc, corrected QT; RO, reverse osmosis; sotalol, *d,l*-sotalol

Introduction

Cardiovascular risk evaluation is a critical component during the safety assessment of new therapeutic agents. During non-clinical drug development, a variety of *in vitro, ex vivo* and *in vivo* models are utilized to identify cardiovascular hazards and perform risk assessment (Guth, 2007; Wallis, 2010). A key step is the conduct of a non-rodent cardiovascular telemetry study to determine the effect of a new agent on haemodynamics, cardiac contractility and electrical activity (ECG). The telemetry method is accepted as the gold standard for measuring cardiovascular function in unrestrained dogs and non-human primates (NHPs), and is especially useful for the detection of drug-induced changes in ventricular repolarization such as corrected QT (QTc) prolongation (Toyoshima *et al.*, 2005; Hanson *et al.*, 2006). The conduct of an *in vivo* QTc evaluation is a required core assay during non-clinical drug development (ICH S7B regulatory guidance; Anon, 2005).

Cardiovascular telemetry is an optimal method for ECG assessment because cardiac endpoints can be captured wirelessly from unstressed animals maintained in their home-cage environment. This is ideal because physiological stress, due to behavioural reactions, environmental factors, human interactions or restraint, can induce tachycardia that confounds the interpretation of QT interval changes and reduces sensitivity (Bass *et al.*, 2009; Guth *et al.*, 2009). While implanted telemetry has many advantages, this powerful method does require surgical implantation of devices, post-operative care of subjects and significant capital investment, including long-term housing costs to enable the re-use of implanted animals.

Recently, an alternative telemetry method has emerged as a new tool for ECG evaluation in large animals: non-invasive or jacket telemetry (Chui *et al.*, 2009; Prior *et al.*, 2009; McMahon *et al.*, 2010). Non-invasive ECG technology offers the opportunity to conduct cardiac drug safety evaluation without the need for surgical implantation of ECG leads and has the potential to be integrated into acute or long-term drug toxicity studies. This latter scenario is valuable for testing therapeutics with unique cardiovascular safety pharmacology needs, such as oncology agents and biotherapeutics (Amouzadeh and Vargas, 2013; Vargas *et al.*, 2013). Like implanted telemetry, jacket telemetry enables the collection of cardiac function data (conduction, repolarization, rate, rhythm, morphology) in unstressed animals and avoids the negative influences of restraint-associated stress (Tattersall *et al.*, 2006, Guth *et al.*, 2009). The current practice of 'snap-shot ECG' measurement in toxicology studies, that is, a few cardiac waveforms collected from restrained/stressed animals at a single time point, may miss or underestimate actual drug-induced changes and have little value in pro-arrhythmia risk assessment (Bass *et al.*, 2009). The use of jacket-based telemetry in toxicity studies has the potential to improve the detection of cardiac electrical and rhythm abnormalities associated with chronic exposure to small molecules and biological agents (Sarazan *et al.*, 2011)

Non-invasive ECG telemetry has already been shown to be an effective approach in dogs (Chui *et al.*, 2009; Prior *et al.*, 2009), but there are no published reports on the use of jacket telemetry for evaluating drug-induced ECG changes in NHPs (Vargas *et al.*, 2010; Derakhchan *et al.*, 2011a,b). Holter monitoring has been used previously for continuous ambulatory ECG and arrhythmia detection in unrestrained monkeys (Macallum and Houston, 1993), but its application in drug safety studies has not been widely adopted. Therefore, this study evaluated the ability of the jacketed external telemetry (JET™) system to detect QTc interval prolongation induced by *d,l*-sotalol (or sotalol) in the NHP. This class III anti-arrhythmic and non-selective β-adrenoceptor antagonist has been used to produce QTc interval prolongation and bradycardia (Derakhchan *et al.*, 1998; Sasaki *et al.*, 2005; Lynch *et al.*, 2008; Ishizaka *et al.*, 2009). This sotalol evaluation included a head-to-head comparison of ECG values derived from implanted telemetry with those derived from JET and collected simultaneously from the same animals. The initial portion of this study focused on the acclimation period needed for NHPs to adjust to jacketing.

Methods

Animals

Twelve male cynomolgus monkeys (*Macaca fascicularis*), 3.4–6.3 years, 3.1–6.5 kg, were cared for in accordance to the *Guide for the Care and Use of Laboratory Animals*, 8th Edition (Institute for Laboratory Animal Research, 2011). Animals were housed individually at an indoor, Association for Assessment and Accreditation of Laboratory Animal Care, international-accredited facility in species-specific housing. All procedures in this study were as humane as possible and complied with the Animal Welfare Act, the *Guide for the Care and Use of Laboratory Animals*, and the Office of Laboratory Animal Welfare. All studies are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Animals were fed a certified pelleted primate diet (PMI #5048, Richmond, IN, USA) daily in amounts appropriate for the age and size of the animals, and had *ad libitum* access to water via automatic watering system. Water samples were routinely analysed for specified microorganisms and environmental contaminants. Environmental controls for the animal room were set to maintain 18–26°C, a relative humidity of 30–70% and a 12 h light/12 h dark cycle. Animals were given additional supplements as a form of environmental enrichment and various cage-enrichment devices.

Conventional surgical methods were used to implant a radiotelemetry transmitter [PCT; Model No. TL11M2-D70-PCT; Data Sciences International (DSI), St. Paul, MN, USA], position ECG leads subcutaneously (lead II configuration) and insert a pressure catheter in the femoral artery. Animals included in this study had been used previously for routine cardiovascular safety pharmacology studies (small molecules; cross-over design) and had a washout (2–4 weeks) before jacket acclimation commenced. Monkeys with normal ECG waveforms and arterial pressure (AP) values, as assessed during the pre-study phase (day –19), were included in the JET evaluation. The JET study was conducted in two phases: jacket acclimation, followed by vehicle or sotalol treatment (Table 1). During the JET study, animals were housed singly and maintained in rooms with standard environmental controls and a 12 h day/12 h night cycle.

Table 1
Study design

Study day	Procedure	Dose group (*n*)	Comment
JET acclimation phase			
–19	–		No jacket (PCT only)
–16			Jacketing†
–15	JET-1	Sham (12)	Haemodynamic collection (24 h)
–14 and –13			No jacket
–12			Jacketing†
–11	JET-2	Sham (12)	Haemodynamic collection (24 h)
–10 and –9			No jacket
–8			Jacketing†
–7	JET-3	Sham (12)	Baseline Haemodynamic and ECG collection (24 h)
Treatment phase			
–1			Jacketing†
1	JET-4	Group 1: Vehicle (6) Group 2: Vehicle (6)	ECG collection (24 h)
2–6			No jacket
7			Jacketing†
8	JET-5	Group 1: Vehicle (6) Group 2: Sotalol 8 mg·kg^{-1} (6)	ECG collection (24 h)
9–13			No jacket
14			Jacketing†
15	JET-6	Group 1: Vehicle (6) Group 2: Sotalol 16 mg·kg^{-1} (6)	ECG collection (24 h)
16–20			No jacket
21			Jacketing†
22	JET-7	Group 1: Vehicle (6) Group 2: Sotalol 32 mg·kg^{-1} (6)	ECG collection (24 h)
23			No jacket

Study design for evaluation of JET in NHPs: schedule of acclimation and treatment activities.
†ECG electrodes and jackets secured (under ketamine sedation) 12 h prior to the 24 h ECG collection.

Jacketing procedure and acclimation period (phase 1)

NHPs were housed in the study room for 2–3 weeks to begin acclimation to oral dosing and jacketing. During acclimation, sham dosing (5 mL·kg^{-1} of water) and jacketing was performed according to a set schedule (Table 1). On the day before each ECG collection (e.g. JET-1 to JET-7), NHPs were anaesthetized with ketamine (0.1 mg·kg^{-1}, i.m.) to enable accurate placement of skin electrodes, confirm ECG waveforms and secure jackets (with telemetry transmitters). The ECG skin electrodes (Vermed Performance Plus Electrode A10012-60S, Bellows Falls, VT, USA) were placed in lead II configuration as follows: white lead on the right of the upper sternum; black lead on the left lower rib cage; and green lead on the right side below the rib cage. During the acclimation phase, each NHP was jacketed three times (Table 1) to assess impact of the process on AP and heart rate (HR) before (day –19) and after jacketing. The last jacketing session (JET-3) was used to establish 24 h baseline HR and QTc values.

ECG evaluation: treatment with vehicle or sotalol (phase 2)

NHPs were divided into two parallel dose groups ($n = 6$) and treated with either vehicle (group 1) or sotalol (group 2). From each group, JET-ECG was collected (24 h) weekly over 4 weeks (Table 1). Vehicle was given on days 1, 8, 15 and 22, and sotalol, as follows: vehicle (day 1), 8 mg·kg^{-1} (day 8), 16 mg·kg^{-1} (day 15) and 32 mg·kg^{-1} (day 22). Vehicle or sotalol was administered by oral gavage [5 mL·kg^{-1}, plus 5 mL reverse osmosis (RO) water flush] and adjusted according to body weight (determined weekly). Animals were fasted overnight prior to dosing, but food was provided after dosing (~5 ± 0.5 h).

A toxicokinetic assessment of sotalol was conducted in a satellite group of six un-instrumented NHPs. Blood samples were collected according to the same sotalol dose paradigm (0, 8, 16 and 32 mg·kg^{-1}) used for the JET evaluation (Table 1). Blood samples (~1 mL; 11 per dose) were collected from a femoral or saphenous vein in lithium heparin tubes, placed immediately on ice, then centrifuged for 10 min at 2000× *g*

(2–8°C) within 1 h of collection. Plasma was collected and frozen at –80°C until analysis. Bioanalysis was conducted by LC-MS with electrospray ionization and multiple reaction monitoring in the positive ion mode. Atenolol was used as the internal standard. The lower limit of quantification of sotalol was 1 ng·mL^{-1}. Toxicokinetic parameters were generated from plasma concentrations using non-compartmental analysis performed on a Watson LIMS (software version 7.0.0.01; Philadelphia, PA, USA).

Data acquisition and analysis

On each ECG collection day (JET-1, JET-2, etc.), ECG data were collected for 90 min before and then 24 h after vehicle or sotalol. Dosing occurred in the morning (~10:00 h) and was completed within 25 min from the end of dosing for the first animal to the end of dosing for the last animal. Haemodynamic and ECG data were separated into day and night cycles. Day cycle data were from pre-dose to approximately 7 h post-dose and night cycle data from 8 to 19 h post-dose. The second light cycle covered data from 20 to 24 h post-dose.

During the acclimation phase, only PCT-derived AP and HR (not ECG) data were assessed for adaptation to repeated jacketing. The pre-dose values were recorded as 1 h averages taken prior to sham dosing. Post-dose data were divided into 15 min (0–7 h) and 1 h averages (8–24 h). Some data collection periods (0–1 and 5–6 h) were excluded from analysis due to sham dosing and husbandry/feeding activities. During the treatment phase, only PCT- and JET-derived ECG intervals and HR values were assessed (e.g. PCT-derived AP was not assessed).

PCT implant data were collected using a DSI RPC-1 receiver, and data from JET system were acquired using a Bluetooth receiver, at 2.4 GHz. DSI Dataquest® OpenART® telemetry equipment was used to generate and acquire the data input. This system transferred the cardiovascular signals to a PONEMAH P3P (Ponemah Physiology Platform, version 4.7) analysis system. The AP and temperature signals were digitized at a sampling rate of 250 Hz, and the ECG signal sampled at 500 Hz. All PCT and JET Ponemah raw data were converted with EMKA ecg-auto (version 2.5.1.18; Paris, France) into *.d01 files for analysis purposes. Outputted data were reported in 300 s bins for the duration of the experiments. Segments of ECG data from each telemetry collection during the dosing phase were used to construct a library of representative ECG waveforms for each animal. ECG waveforms that matched the library were analysed to determine ECG parameters, including PR, QT and rate-corrected QT (QTc, using the Bazett's correction; Bazett, 1920; Soloviev *et al.*, 2006) intervals and QRS duration.

Statistical evaluation

All cardiovascular and pharmacokinetic values are presented as mean ± SEM. Further analysis is described below, for each phase.

Phase 1. During acclimation, PCT-derived AP and HR values were compared before (day –19) and after each jacketing session (days –15, –11 and –7). For comparison of pre-jacketed and jacketed AP and HR values, a mixed effect model was applied, and time and condition effects were included as covariates. Treatment comparisons were assessed with Dunnett's *t*-test.

Phase 2. During treatment phase, data collected by both PCT and JET telemetry were analysed separately. Group 1 and 2 data sets were analysed by analysis of covariance with repeated measures (time), according to the treatment day of the study (days 8, 15 and 22). Day 1 values (for each ECG parameter) were used as the covariate for each subject. Dunnett's (or Dunnett-Hsu) *t*-test was used for group comparisons to assess treatment or dose effects, and each treatment group was compared with its corresponding vehicle-treated group. Statistical significance was accepted at a $P < 0.05$.

Comparison of JET- and PCT-derived values. To determine the equivalence of the cardiac interval and HR values measured by JET and PCT, a mixed effect model was applied to the log-transformed data with collection technology and time as fixed effects. Mixed effect model methodology is often applied to analyse experiments where there are multiple sources of variability. We have used this analysis to accommodate this experimental design taking into consideration the correlation of measurements taken on the same animal over time. Validity of the model was evaluated using the histogram and the normal probability plot of the residuals. The logarithmic data transformation enabled a linear regression correlation analysis of JET versus PCT data. The difference between JET and PCT values, and its two-sided 90% confidence interval, was estimated and transformed back to the original scale [ms or beats per minute (bpm)] to report the ratio of the geometric means of the two methods and the corresponding two-sided confidence interval. *P*-value of the difference was also reported. Equivalence analyses (SAS, 1999; v9.2) were conducted only for NHP treated with vehicle or escalating doses of sotalol (days 1, 8, 15 and 22). The geometric means were estimated by exponentiating the least square mean estimate of the log-transformed data points.

Materials

The vehicle control was 0.5% (w/v) hydroxypropyl methylcellulose (HPMC, Lot No. XQ1146; Spectrum Laboratory Product Inc., New Brunswick, NJ, USA) in RO water. Sotalol (Lot No. 087K4065; Sigma-Aldrich, St. Louis, MO, USA) was prepared in 0.5% (w/v) HPMC in RO water.

Results

Impact of jacketing on HR and AP

During the acclimation phase, 12 NHPs were jacketed three times, and AP and HR values were measured over 24 h to assess the impact of jacketing (Table 1). PCT-derived AP and HR values obtained from non-jacketed animals were used as the reference point (day –19). In non-jacketed NHPs, HR demonstrated normal circadian variation, with lower HR values observed during the night cycle compared with day cycle (day: 118 ± 6 bpm vs. night: 92 ± 4 bpm). In NHP wearing jackets for the first time (JET-1), there was no significant difference in PCT-derived HR values compared with non-jacket values (day: 121 ± 6 bpm vs. night: 93 ± 6 bpm). On subsequent jacket sessions (JET-2 and JET-3), the HR values continued to exhibit a normal diurnal pattern.

Similar to HR, systolic, diastolic and mean AP exhibited a normal circadian pattern in non-jacketed NHP, with lower values observed at night. The normal circadian pattern of systolic AP was not observed during the first jacketing session (JET-1; day cycle: 107 ± 3 mmHg and night cycle: 107 ± 4 mmHg) when compared with non-jacket values (day cycle: 98 ± 5 mmHg and night cycle: 92 ± 5 mmHg), and the night cycle systolic AP is significantly higher (15 ± 6 mmHg). Diastolic and mean AP were also elevated at night, but did not attained statistical significance. On subsequent jacketing sessions (e.g. JET-2 and JET-3), systolic AP values remained slightly higher during day (6–8 mmHg) and night cycles (9 mmHg), but demonstrated a diurnal pattern. The NHPs acclimated to the jackets during the second and third jacket sessions, as night time AP values approached non-jacket values. These findings indicated that the jacket acclimation process implemented for this study was appropriate to condition the NHP and establish normal (baseline) day and night cardiovascular values.

ECG evaluation in vehicle-treated animals

JET-derived HR data from vehicle-treated animals were similar to PCT-derived HR values (Figure 1). In addition, both JET and PCT were capable of capturing very consistent QTc intervals following repeated administration of vehicle in the same animals, over 4 weeks of evaluation (Figure 2).

JET-derived HR and QTc interval values obtained after sham dosing on the last day of jacket acclimation (JET-3; day –7) were similar to JET-derived HR and QTc interval data obtained after vehicle treatment (JET-4; day 1, data not shown). This last jacket acclimation session was used as the baseline reference point for all comparisons to treatment.

ECG evaluation in sotalol-treated animals

JET produced high-quality ECG waveforms in conscious NHPs that were comparable to PCT-derived waveforms. In Figure 3, a review of ECG waveforms from a single monkey given 32 mg·kg^{-1} sotalol demonstrated QT interval prolongation with both telemetry systems. The morphological change in T-wave end is evident and consistent with sotalol T_{max}. These were observed in all group 2 NHPs treated with sotalol.

Peak plasma sotalol concentrations appeared 1–3 h after dosing and dose-proportional increases in mean C_{max} (total) were observed. Concentrations of 4660 ± 180, 7400 ± 1100 and 17 900 ± 810 ng·mL^{-1} were attained after 8, 16 and 32 mg·kg^{-1} p.o. respectively. Drug levels declined slowly and were still detectable 24 h after dosing.

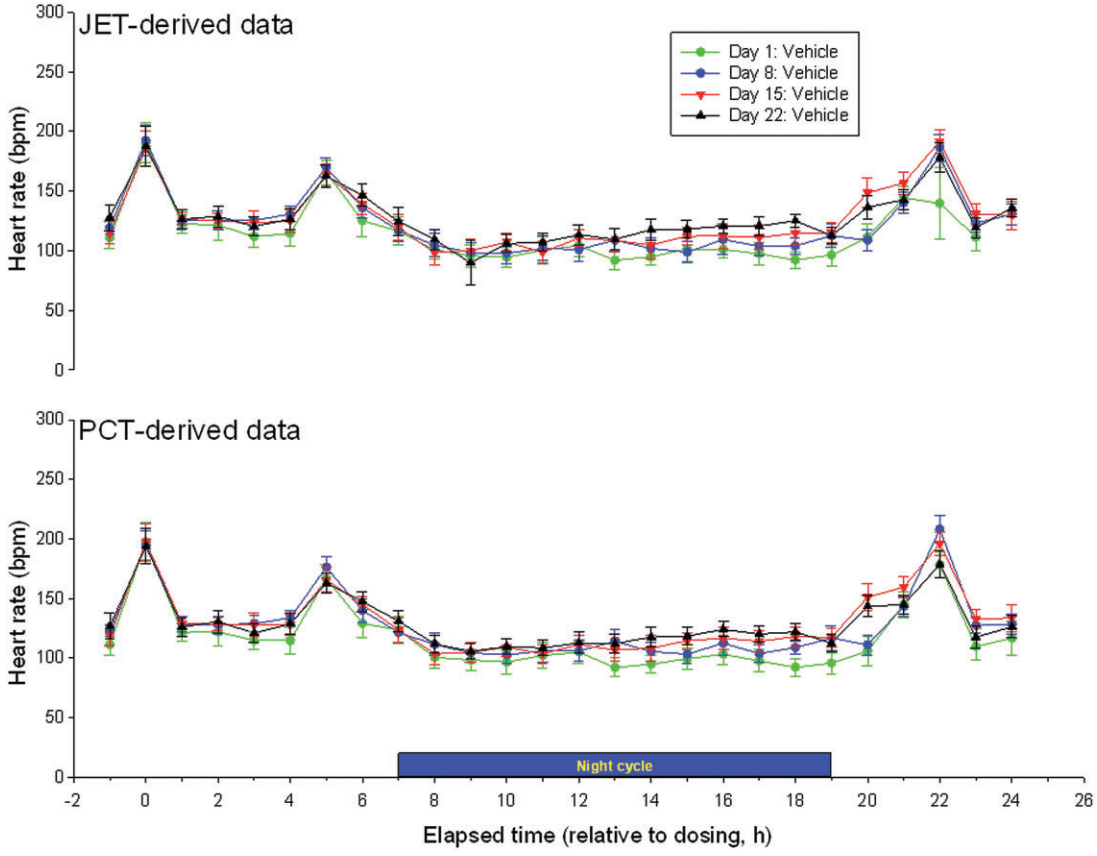

Figure 1

Time course of heart rate derived from JET (top panel) and PCT (bottom panel) from the same group 1 animals following vehicle administration. Data are 1 h averages and are expressed as mean ± SEM. Heart rate elevations at 0–1 h are due to oral gavage, at 5–6 and 21–22 h are responses to room entry.

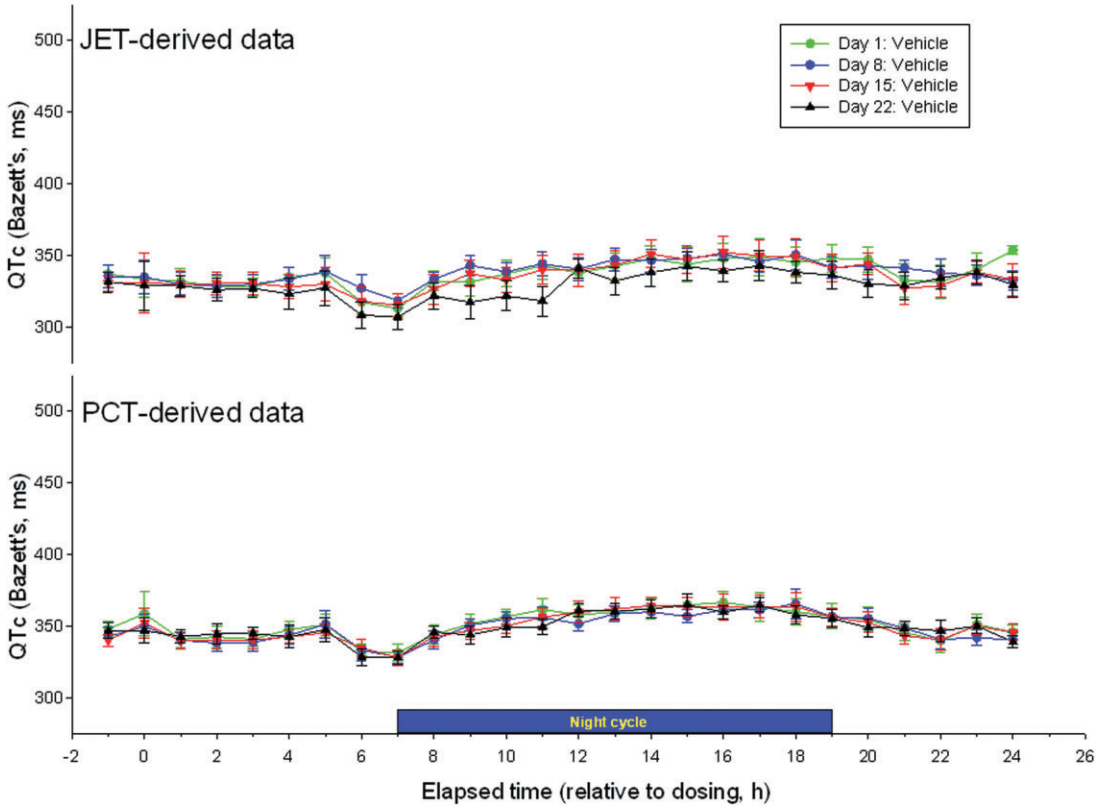

Figure 2

Time course of QTc interval derived from JET (top panel) and PCT (bottom panel) from the same group 1 animals following vehicle administration. Data are 1 h averages and are expressed as mean ± SEM. QTc interval changes at 0–1 h are due to oral gavage, at 5–6 and 21–22 h are responses to room entry.

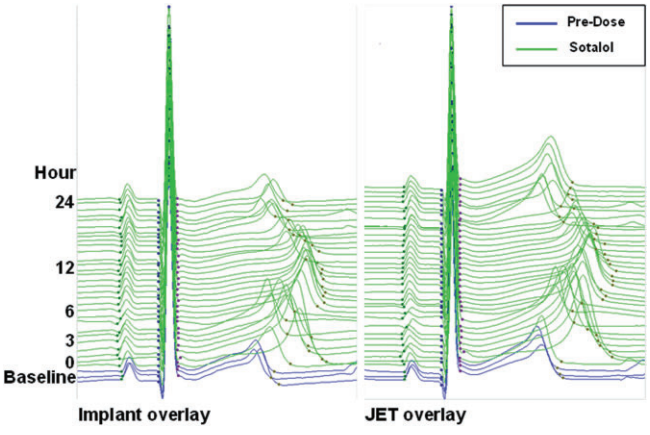

Figure 3

Averaged ECG lead II waveforms over 24 h of a single animal (left panel: implant overlay; right panel: JET overlay) dosed orally with sotalol at 32 mg·kg^{-1}. Each waveform is derived from a 300 s data bin, and time references are relative to dosing. The blue waveform was derived from the baseline, pre-dose period. Cardiac waveforms were aligned on the peak of the R-wave to standardize the identification of the T-wave. Waveforms were generated as a statistical summary by EMKA ecgAuto.

HR, QT and QTc intervals

JET-derived HR values were similar to PCT-derived HR data after treatment with vehicle or sotalol (Figure 4). Administration of escalating doses of sotalol caused HR reduction, but the bradycardia was not dose-dependent (Table 2). In Figure 4, the HR elevation due to room entry at 5–6 h post-dose was almost completely blunted by escalating doses of sotalol. Significant HR effects measured by JET after sotalol (range: from –18 to –43 bpm) were on the same order of magnitude as HR effects measured by PCT (range: from –19 to –54 bpm) when compared with time-matched group 1 animals.

As shown in Table 2, significant prolongation of QT and QTc intervals could be accurately and consistently detected using JET, after sotalol treatment. JET-derived QTc intervals after sotalol were very similar to the absolute QTc time intervals measured by PCT implant (Figure 5). Significant QT interval prolongation ranged from 30 to 134 ms, as calculated by the absolute difference in covariate-adjusted mean of sotalol- (group 2) and vehicle-treated animals (group 1). Significant QTc interval prolongation ranged from 14 to 118 ms and directly varied by sotalol dose and time after treatment.

PR interval and QRS duration

As shown in Figure 6 and Table 2, JET detected accurately PR interval prolongation to the same degree as with PCT

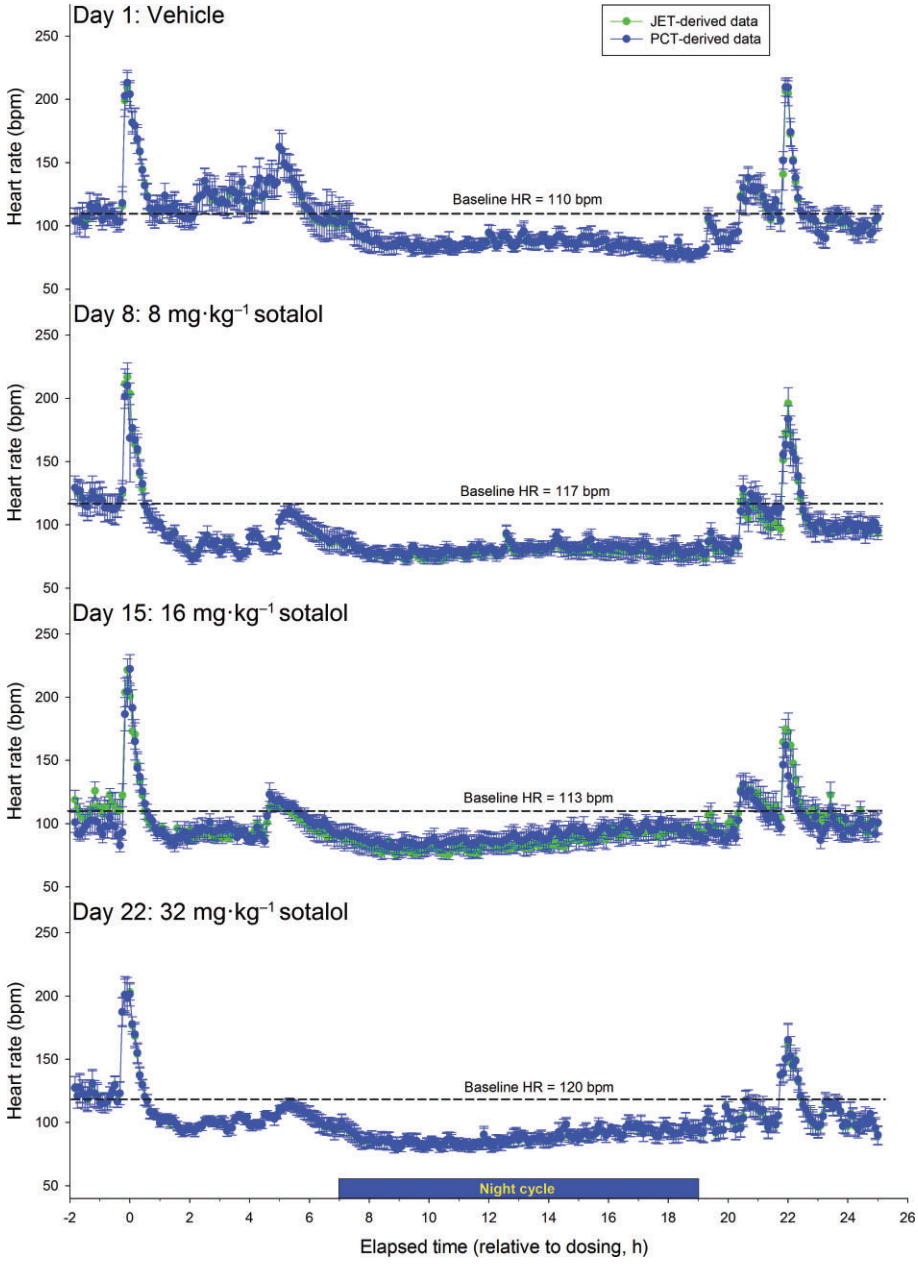

Figure 4

Time course of HR values derived from JET and PCT from animals in the sotalol-treated group, measured over 4 weeks. Each point represents a 5 min data bin over the pre-dose and post-dose time course after dosing with vehicle (day 1) and escalating doses of sotalol (days 8–22). Data expressed as mean ± SEM (n = 6).

implants. PR interval widening was observed consistently with both JET and PCT in NHPs given graded doses of sotalol, but the effect was not dose-dependent. The PR effect followed the same HR dose–response pattern and may be a secondary response attributed to bradycardia. Significant PR interval prolongation detected in this study ranged from 4 to 12 ms, calculated as the absolute difference in the covariate-adjusted mean of animals given sotalol (group 2) when compared with vehicle-treated animals (group 1).

As shown in Figure 7, JET was able to measure QRS duration that was very close to values measured with PCT implants. The QRS interval appeared to increase (≤10 ms) following the middle and high doses of sotalol in both the JET and the PCT groups, but these changes were not statistically significant compared with baseline values.

ECG morphology

Multiple patterns of atrial and junctional premature complexes, ventricular premature complexes, bigeminy, accelerated idioventricular rhythm and ventricular tachycardia were observed in animals given 16 or 32 mg·kg^{-1} sotalol. The

Table 2

Peak sotalol effect detected by JET and PCT

Dose level (mg·kg^{-1})	ECG system	Heart rate (bpm)	PR (ms)	QRS (ms)	QT (ms)	QTc (ms)
Pre-dose (absolute values)	JET	115 ± 2	74 ± 0.2	39 ± 1	248 ± 2	339 ± 2
	PCT	115 ± 2	74 ± 0.5	42 ± 1	246 ± 2	337 ± 1
Absolute peak differences from vehicle-treated animals (group 1)						
8	JET	–37*	12**	NS	95**	27*
	PCT	–54**	9*	NS	98**	44**
16	JET	–32*	9*	NS	84*	54*
	PCT	–32*	6*	NS	78*	54*
32	JET	–43**	5*	NS	120**	76*
	PCT	–47**	5*	NS	134**	118**

Comparison of heart rate and ECG changes measured by JET and PCT after sotalol treatment.
The peak effects were calculated as the absolute difference in covariate-adjusted mean of sotalol- (group 2) and vehicle-treated animals (group 1). Pre-dose values are the grand mean (1 h average) for each parameter measured on days 1, 8, 15 and 22 in group 2. The data are absolute values expressed as mean ± SEM.
*$P < 0.05$ and **$P < 0.01$ statistically significant from animals in group 1 ($n = 6$) when *Treatment* × *Time* was significant.
NS, non-significant.

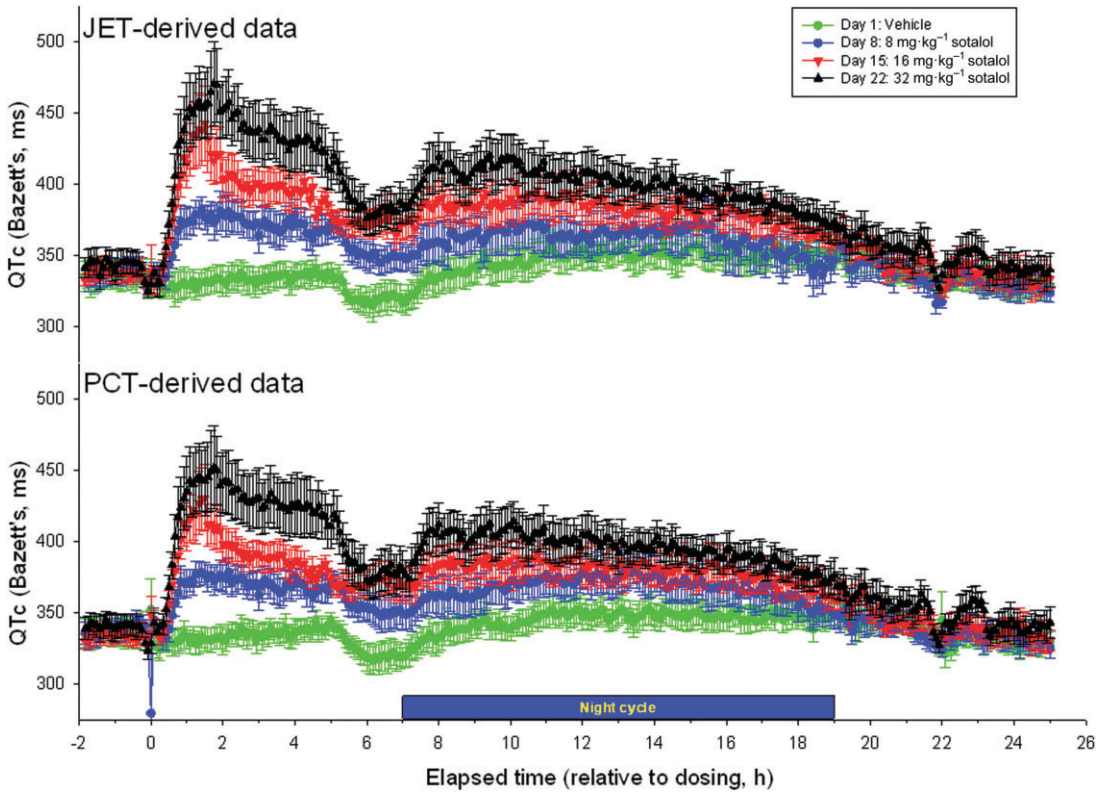

Figure 5

Time course of QTc interval derived from JET (top panel) and PCT (bottom panel) from the same group 2 animals, following administration of vehicle, or 8, 16 and 32 mg·kg^{-1} sotalol. Each point represents a 5 min data bin over the pre-dose and post-dose time course. Data expressed as mean ± SEM.

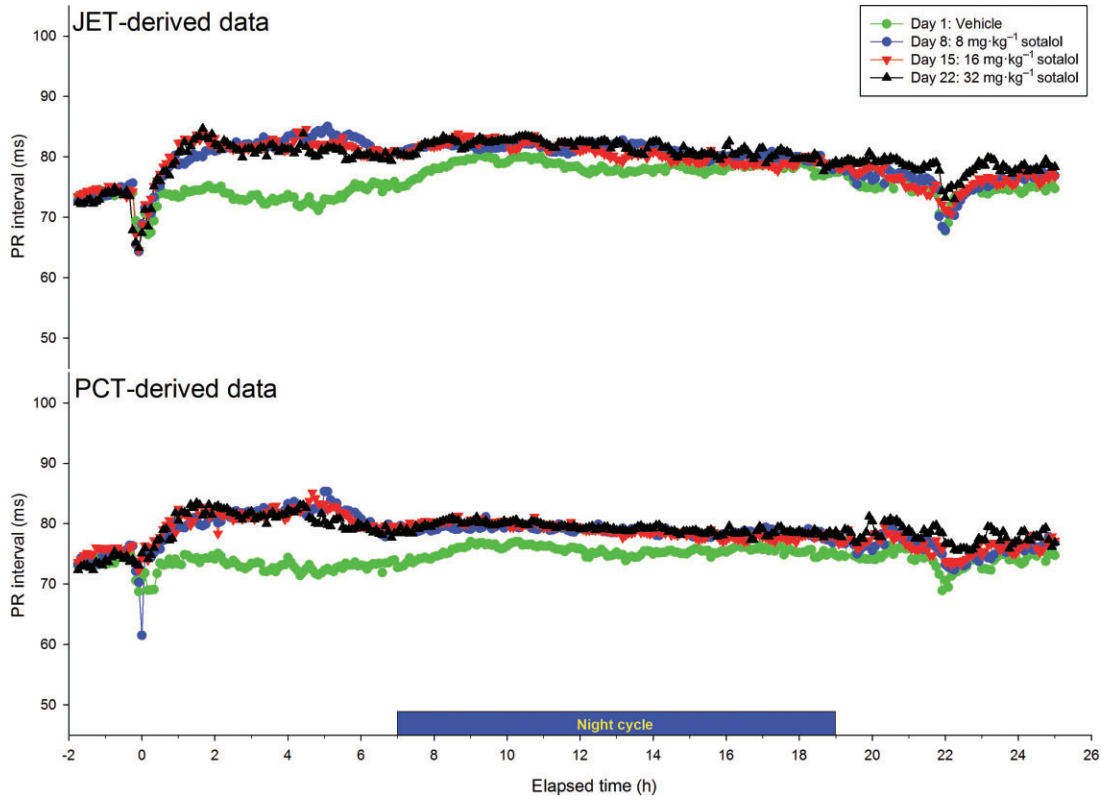

Figure 6

Time course of PR interval derived from JET (top panel) and PCT (bottom panel) from the same group 2 animals, following administration of vehicle, or 8, 16 and 32 mg·kg^{-1} sotalol. Each point represents a 5 min data bin over the pre-dose and post-dose time course.

incidence and severity of these arrhythmias scaled with dose (not shown) and were attributed to sotalol treatment.

ECG data: equivalence between invasive and non-invasive technologies

The equivalence between the absolute QT, QTc and PR intervals, QRS duration and HR values derived from each ECG technology is shown in Figure 8 and Table 3. These scatter plots show the correlation of values obtained by the two ECG systems, and the data for each endpoint and dose of sotalol are presented on separate graphs. For HR, QT and QTc intervals, the statistical comparison of JET and PCT demonstrated excellent correlation, with almost all the data points on the equity line. For the PR interval, the values measured by JET tended to be slightly higher (on average) than values derived by PCT, but the difference was relatively small (<1.9% or 1 ms; see Table 3) on the four data collection days. Absolute QRS duration measurements made with JET tended to be slightly smaller than values measured by PCT, with JET-QRS values being 10, 5 and 4% lower on days 1, 8 and 15 respectively (Table 3). The *P*-values observed for the difference in cardiac interval and HR data between the two ECG technologies were all very small, and the geometric mean ratios for JET/PCT values approximated 1 (Table 3), which indicated that JET-derived values were almost identical to values obtained with PCT.

Discussion and conclusion

The purpose of this study was to test the ability of the JET system to quantify basal and drug-induced changes in ECG intervals and HR in the NHP. A key component of the validation study included a head-to-head comparison of sotalol-induced QTc prolongation using both non-invasive JET and standard telemetry implants (PCT) in the same animals. The findings from this study clearly demonstrate, for the first time, that JET can be used to conduct high-quality ECG collections in the cynomolgus monkey, and that this non-invasive ECG approach is sensitive and can be used to detect QTc prolongation. This work builds upon, and directly extends, our past experience with the use of JET in dogs (Chui *et al.*, 2009).

A total of seven jacket placements and removals (three during acclimation phase and four during treatment) were conducted over 6 weeks, and all animals tolerated the repeated application of skin electrodes and jackets, and no test subjects dropped out of the study. The study design for the JET validation was chosen to mimic a 4 week toxicity study (two parallel dose groups) with weekly ECG collection. An important facet of this study was getting the animals acclimated to the jackets during the pre-study period. The cynomolgus monkeys exhibit natural circadian variations in AP and HR, when tracked by implanted telemetry (Gauvin

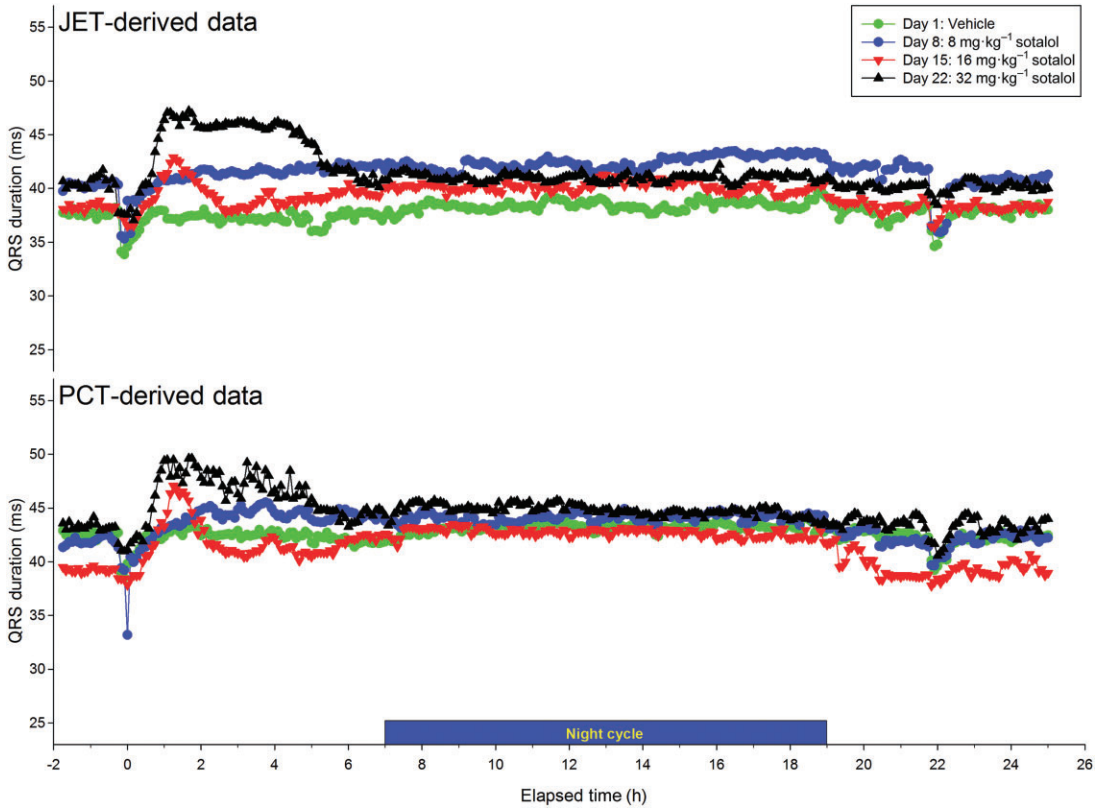

Figure 7

Time course of QRS duration derived from JET (top panel) and PCT (bottom panel) from the same group 2 animals, following administration of vehicle, or 8, 16 and 32 mg·kg^{-1} sotalol. Each point represents a 5 min data bin over the pre-dose and post-dose time course.

et al., 2005). In the current study, unjacketed NHPs also showed a circadian pattern, with lower HR and systolic AP values at night (HR-day: 118 ± 6 bpm vs. night: 92 ± 4 bpm; systolic AP-day: 98 ± 5 bpm vs. night: 92 ± 5 mmHg). Acclimation of NHPs to jacket telemetry was not associated with obvious effects on absolute HR values, or diurnal changes in HR, which indicated that NHPs acclimated very quickly to jacketing (36 h × 3 sessions) and had no signs of physiological stress or behavioural agitation while wearing jacket telemetry.

With regard to AP, initial jacketing caused a significant elevation in systolic AP, and dampened the normal circadian pattern; however, pressure did trend towards non-jacket values and reach steady state by the second collection. A potential explanation for the slight elevation in AP observed on the second and third jacketing sessions, despite the apparent onset of acclimation based on the HR data (above), is that tissue compression or restricted movement caused by the jacket could be factors that indirectly affect AP in the NHP. Because general animal activity was not measured and video-tracking of animal behaviour was not employed, additional work is needed to explore the potential impact of these factors. Based on the observations of the current study, the jacket acclimation process (minimum of two jacket sessions) was appropriate to condition the animals, and yield normal (physiological) day and night cardiovascular values in this species. It should be recognized, however, that the period required to complete jacket acclimation could vary and be influenced by the source, age, size, gender and temperament of the NHP being used, so the jacket acclimation regimen could differ between animals.

In jacketed NHPs, sotalol produced dose-related increases in QT and QTc intervals and reduced HR as expected, based on the pharmacological properties of this agent. The absolute cardiac interval and HR values were generally consistent between PCT telemetry and JET, which indicates that the non-invasive ECG method was able to reliably quantify ECG intervals in conscious monkeys. From a sensitivity perspective, the magnitude of actual sotalol-induced QTc prolongation and bradycardia determined by the JET method was nearly identical to the peak effects estimated by PCT. This positive correlation reinforces the proposition that JET can accurately detect changes in ventricular repolarization function as effectively as implanted telemetry which is considered the gold standard, and that JET is a valid tool for ECG evaluation in this animal model. On a technical note, as the ECG lead placements for PCT and JET were placed in different locations (surface vs. subcutaneous), and slightly different locations relative to the heart, small differences in absolute cardiac intervals were expected. However, these small time differences did not affect interpretation of cardiovascular responses to sotalol.

Like the QT/QTc data set, JET-derived PR and QRS intervals showed good trend agreement with those derived from PCT. The minor discrepancies between JET and PCT implants

Table 3

Equivalence of HR and cardiac interval values measured by JET and PCT

Parameter	Day	Vehicle (V) or sotalol (mg·kg^{-1})	Geometric mean of JET	Geometric mean of PCT	JET/PCT ratio	LB of 90% CI	UB of 90% CI
HR (bpm)	1	V	100	100	0.997	0.996	0.997
	8	8	90	90	0.997	0.997	0.998
	15	16	94	94	0.996	0.996	0.997
	22	32	98	98	0.997	0.996	0.997
PR (ms)	1	V	75	74	1.019	1.018	1.021
	8	8	79	78	1.013	1.011	1.015
	15	16	79	78	1.009	1.007	1.010
	22	32	79	78	1.016	1.014	1.017
QRS (ms)	1	V	38	42	0.891	0.889	0.893
	8	8	41	43	0.949	0.946	0.951
	15	16	39	41	0.959	0.956	0.962
	22	32	43	43	0.992	0.990	0.994
QT (ms)	1	V	261	260	1.001	1.000	1.002
	8	8	287	288	0.998	0.997	0.999
	15	16	293	291	1.007	1.006	1.008
	22	32	299	296	1.009	1.008	1.010
QTc (ms)	1	V	338	338	0.999	0.998	1.000
	8	8	353	355	0.995	0.994	0.996
	15	16	368	367	1.004	1.003	1.005
	22	32	384	381	1.008	1.007	1.009

Comparison of 24 h ECG and HR values measured by JET and PCT after sotalol treatment (group 2).
Values are the grand mean of all the JET- and PCT-derived data points following treatment with vehicle or sotalol on each day of the study.
The ratio of 1 indicates uniformity of values attained by the JET and PCT methods.
CI, confidence interval; LB, lower bound; UB, upper bound.

in absolute QRS duration may be due to potential morphological differences in ventricular waveforms obtained from the different leads. Overall with JET, the ECG signal was more prone to artifactual noise and is likely to reflect a limitation of any ECG technology that employs surface electrodes, as these are more susceptible to movement (muscle) artefacts, as well as skin electrode migration or dry out (Vogel *et al.*, 1991; Chui *et al.*, 2009; Guth *et al.*, 2009), and their placement may vary slightly between measurements. Another important factor may be the low amplitude (voltage) of some components of the ECG in monkeys, such as the P-wave (Vogel *et al.*, 1991; Gauvin *et al.*, 2005).

Overall, ECG can be accurately recorded using a non-invasive jacket-based ECG method in NHP, and changes in cardiac intervals (PR, QRS and QT) and in heart rhythm could be detected after drug treatment. Jacketed animals are unstressed based on HR evaluation (compared with toxicological study animals stressed by physical or chemical restraint for ECG collection), which results in higher quality ECG data obtained over long time intervals, for example, 24 h. Optimization of technical aspects, jacket acclimation, ECG library construction and data analysis methods are other factors that can be maximized to improve JET data quality and sensitivity to detect drug-induced changes in cardiac conduction and repolarization.

In conclusion, this study confirmed that the JET system for ECG is valid for cardiac interval and HR monitoring in the primate and can yield reproducible, high-quality data. The method is capable of detecting QTc prolongation induced by sotalol and is a valuable non-invasive tool that can be used for non-clinical cardiovascular safety assessment. The JET approach can be used to collect high-fidelity ECG data in short- and long-term NHP studies of 1–6 months duration (Vargas *et al.*, 2010; Chui *et al.*, 2011; Derakhchan *et al.*, 2011b). The use of jacket telemetry is a good example of how to integrate cardiovascular safety pharmacology endpoints into toxicity studies (Luft and Bode, 2002; Redfern *et al.*, 2013; Vargas *et al.*, 2013). A limitation of jacket-based ECG is its inability to collect BP simultaneously (McMahon *et al.*, 2010), which is important for assessing drug-induced alterations in vascular function and haemodynamics.

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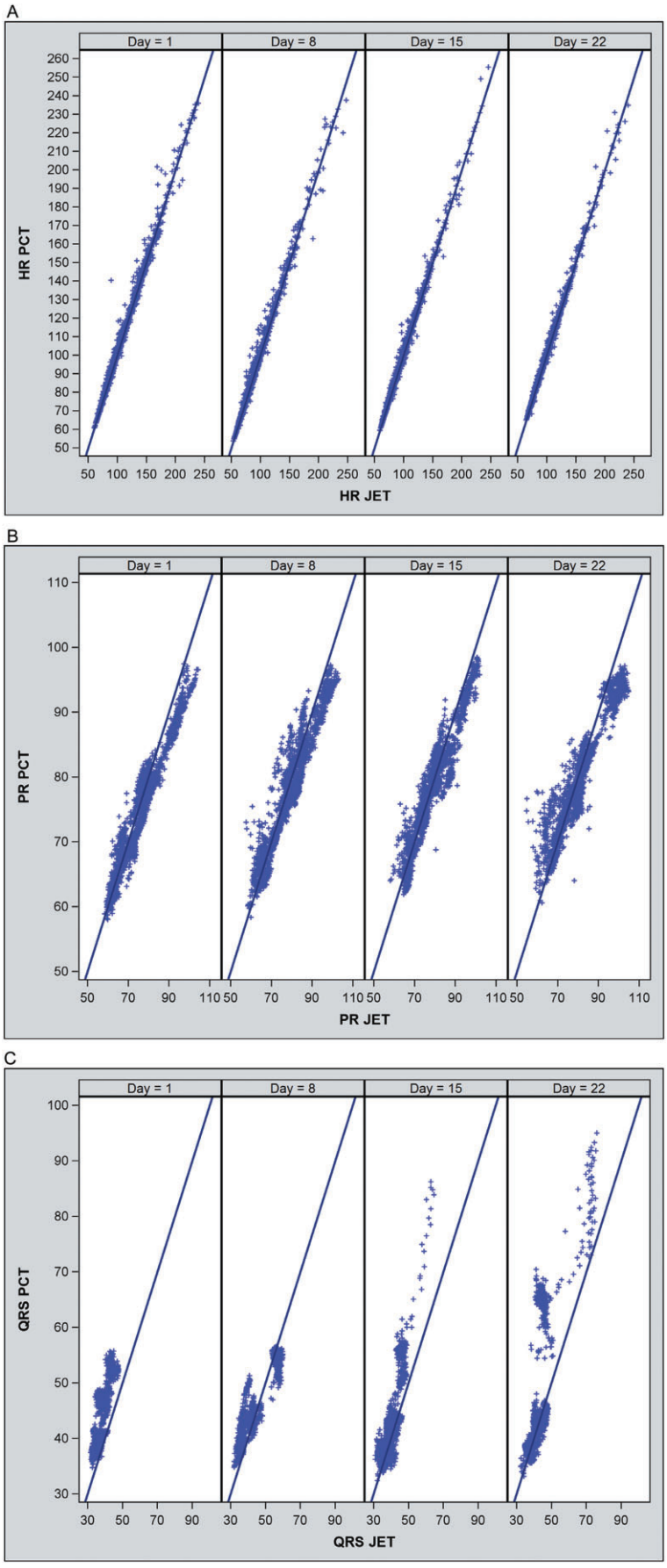
A
Day = 1
Day = 8
Day = 15
Day = 22
HR PCT
HR JET
B
PR PCT
PR JET
C
QRS PCT
QRS JET

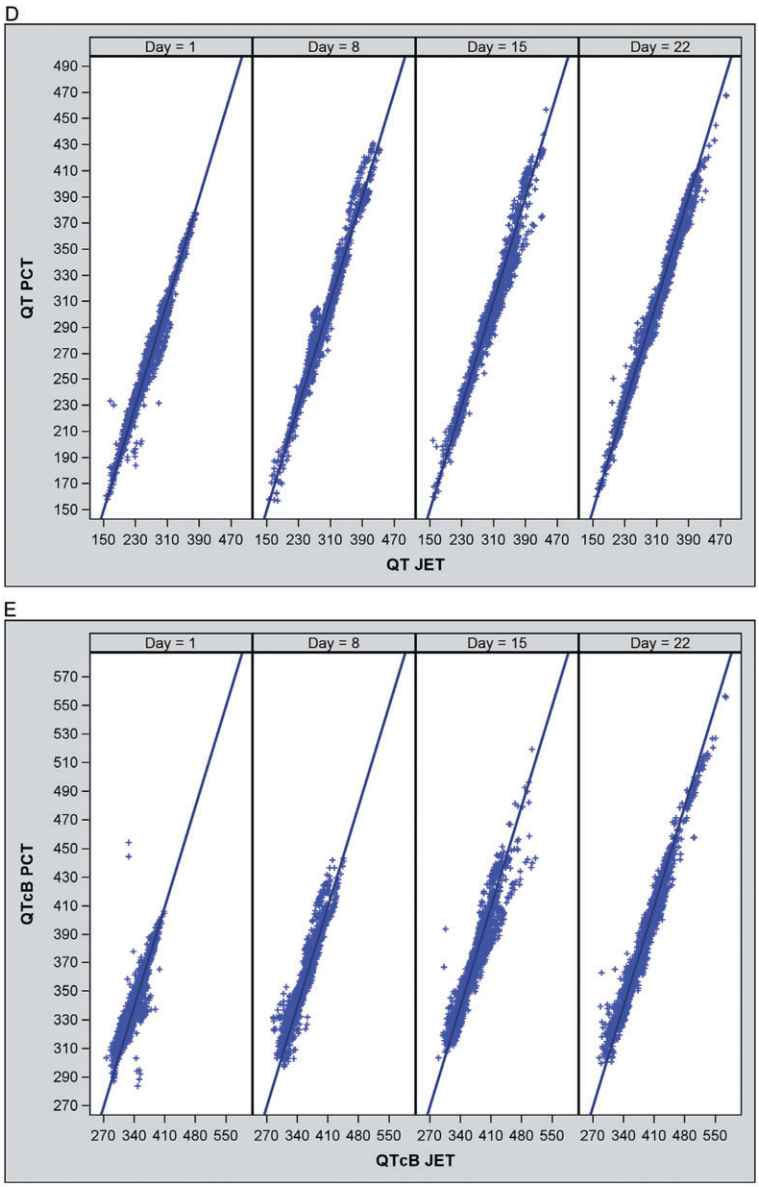

Figure 8

Equivalence of the responses generated by JET and PCT technologies – scatter plots of PCT ECG endpoints versus JET ECG endpoints. Each point represents a 5 min data bin over the pre-dose and post-dose time course (~28 h) after dosing with vehicle (day 1) or escalating doses of sotalol (days 8–22) for a total of six NHPs. Each plot is composed of 1992 data pairs: (A) heart rate (bpm); (B) PR interval (ms); (C) QRS duration (ms); (D) QT interval (ms); and (E) QTc interval (ms).

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Conflict of interest

K Derakhchan, RW Chui and HM Vargas are current employees, whereas D Stevens and W Gu are former employees, of Amgen, Inc. The cardiovascular portion of the study was conducted at Covance Laboratories Inc. (Madison, WI, USA) with financial support from Amgen Inc.

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